

APPLICANTS: Comb *et al.*
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Listing of Claims

- 1: (withdrawn) A method for producing a motif-specific, context-independent antibody that recognizes a plurality of peptides or proteins within a genome that contain the motif, said method comprising the steps of:
 - (a) constructing a degenerate peptide library comprising (i) a fixed target motif comprising one or more invariant amino acids, and (ii) a plurality of degenerate amino acids flanking said motif;
 - (b) immunizing a host with said peptide library to raise a context-independent antibody specific for a motif consisting of all or part of said target motif; and
 - (c) isolating antisera from said host, and purifying the motif-specific, context-independent antibody of step (b) from said antisera, said antibody recognizing a plurality of peptides or proteins within a genome that contain said motif.
2. (previously presented) The method of claim 3, further comprising the step of utilizing spleen cells from the host of step (b) to generate at least one monoclonal, motif-specific, context-independent antibody.
3. (previously presented) A method for producing a motif-specific, context-independent antibody that specifically binds a recurring, modified motif in a plurality of peptides or proteins within an organism in which it recurs, said method comprising the steps of:
 - (a) constructing a degenerate peptide library comprising (i) a fixed target motif comprising two to six invariant amino acids including at least one modified amino acid, and, optionally, one or more degenerate amino acid position(s), and (ii) a plurality of degenerate amino acids flanking said motif;
 - (b) immunizing a host with said peptide library to raise a context-independent antibody specific for all or part of said target motif; and
 - (c) isolating antisera from said host, and purifying the motif-specific, context-independent antibody of step (b) from said antisera, said antibody specifically binding said motif in a plurality of peptides or proteins within an organism in which it recurs.

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4. (previously presented) The method of claim 3, wherein said modified amino acid is a selected from the group consisting of a phosphorylated amino acid, an acetylated amino acid, a methylated amino acid, and a nitrosylated amino acid.
- 5-10. (cancelled).
11. (currently amended) The method of claim 3, wherein said target motif comprises all or part of a kinase consensus substrate motif ~~or a protein-protein binding motif~~.
12. (currently amended) The method of claim 11, wherein said kinase consensus substrate motif is selected from the group consisting of a MAPK consensus substrate motif, a CDK consensus substrate motif, a PKA consensus substrate motif, an AKT consensus substrate motif, a PKC consensus substrate motif, and an ATM consensus substrate motif, ~~and wherein said protein-protein binding motif is selected from the group consisting of a 14-3-3 consensus binding motif, and a PDK1 bulky ring consensus docking motif~~.
13. (currently amended) The method of claim 11, wherein said kinase consensus substrate motif is selected from the group consisting of a PKC Zeta consensus substrate motif, an ABL kinase consensus substrate motif, a CDK5 consensus substrate motif, an insulin receptor consensus substrate motif, a CaMKII consensus substrate motif, a Src kinase consensus substrate motif, a CDC2/CDK2 consensus substrate motif, and a GSK3 kinase consensus substrate motif, ~~and wherein said protein-protein binding is a PI3K P85 consensus binding motif~~.
14. (original) The method of claim 3, wherein said target motif is phosphothreonine-X-(R/K) or proline-phosphoserine-proline.
15. (previously presented) The method of claim 3, wherein said peptide library is from 6 to 20 amino acids long.
16. (previously presented) The method of claim 3, wherein said peptide library is from 6 to 14 amino acids long.
17. (withdrawn) A method for producing a motif-specific, context-independent antibody that recognizes a plurality of peptides or proteins within a genome that contain the motif, said method comprising the steps of:

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- (a) constructing a degenerate peptide library comprising (i) a fixed target motif comprising one to six invariant amino acids, and (ii) a plurality of degenerate amino acids flanking said motif;
 - (b) immunizing a host with said peptide library to raise a context-independent antibody specific for a motif consisting of all or part of said target motif; and
 - (c) isolating antisera from said host, and purifying the motif-specific, context-independent antibody of step (b) from said antisera, said antibody recognizing a plurality of peptides or proteins within a genome that contain said motif.
18. (withdrawn) A method for producing a motif-specific, context-independent antibody that recognizes a plurality of peptides or proteins within a genome that contain the motif, said method comprising the steps of:
- (a) constructing a degenerate peptide library comprising (i) a fixed target motif comprising one to six invariant amino acids including at least one modified amino acid, and (ii) a plurality of degenerate amino acids flanking said motif, wherein said peptide library is from 6 to 20 amino acids long;
 - (b) immunizing a host with said peptide library to raise a context-independent antibody specific for a motif consisting of all or part of said target motif; and
 - (c) isolating antisera from said host, and purifying the motif-specific, context-independent antibody of step (b) from said antisera, said antibody recognizing a plurality of peptides or proteins within a genome that contain said motif.
19. (withdrawn) A motif-specific, context-independent antibody produced by the method of any one of claims 1-3 and 17-18.
20. (withdrawn) A motif-specific, context-independent antibody which recognizes a plurality of peptides or proteins within a genome that contain said motif, wherein said motif comprises one to six invariant amino acids.
21. (currently amended) A motif-specific, context-independent antibody that specifically binds a recurring, phosphorylated motif ~~comprising~~ consisting of (i) two to six invariant amino acids including at least one phosphorylated amino acid, and, optionally, (ii) one or more degenerate amino acid position(s).

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said antibody specifically binding said motif in a plurality of non-homologous peptides or proteins within an organism in which it recurs.

22. (withdrawn).

23. (previously presented) The antibody of claim 21, wherein said motif consists of all or part of a kinase consensus substrate motif or a protein-protein binding motif.

24. (currently amended) The antibody of claim 23, wherein said kinase consensus substrate motif is selected from the group consisting of a MAPK consensus substrate motif, a CDK consensus substrate motif, a PKA consensus substrate motif, an AKT consensus substrate motif, a PKC consensus substrate motif, and an ATM consensus substrate motif, ~~and wherein said protein-protein binding motif is selected from the group consisting of a 14-3-3 consensus binding motif, and a PDK1/bulky ring consensus docking motif.~~

25. (currently amended) The antibody of claim 23, wherein said kinase consensus substrate motif is selected from the group consisting of a PKC Zeta consensus substrate motif, an ABL kinase consensus substrate motif, a CDK5 consensus substrate motif, an insulin receptor consensus substrate motif, a CaMKII consensus substrate motif, a Src kinase consensus substrate motif, a CDC2/CDK2 consensus substrate motif, and a GSK3 kinase consensus substrate motif, ~~and wherein said protein-protein binding motif is a PI3K-P85 consensus binding motif.~~

26. (original) The antibody of claim 21, wherein said motif is phosphothreonine-X-(R/K) or proline-phosphoserine-proline.

27. (withdrawn) A method for identifying an unknown substrate of an enzyme, said method comprising the steps of:

(a) generating at least one motif-specific, context-independent antibody which recognizes a motif common to a plurality of substrates of said enzyme within a genome; and

(b) screening a target sample with the antibody of step(a) to identify an unknown substrate containing said motif.

28. (withdrawn) A method for detecting the modification state of a target substrate that contains a motif common to a plurality of substrates of an enzyme within a genome, said method comprising the

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steps of:

(a) generating at least one motif-specific, context-independent antibody which recognizes the modified form of said motif; and

(b) screening a target sample with the antibody of step(a) for the presence of target substrate containing said modified motif, thereby detecting the modification state of said substrate.

29. (withdrawn) A method for screening a drug for the inhibition or activation of enzyme activity on at least one substrate that contains a motif common to a plurality of substrates of said enzyme within a genome, said method comprising the steps of:

(a) generating at least one motif-specific, context-independent antibody which recognizes the modified form of said motif; and

(b) screening a target sample treated with said drug with the antibody of step(a) to identify substrate(s) containing said modified motif, thereby screening said drug for inhibition or activation of enzyme activity.

30. (withdrawn) A method for identifying an enzyme which modifies a known substrate that contains a motif common to a plurality of substrates of said enzyme within a genome, said method comprising the steps of:

(a) generating at least one motif-specific, context-independent antibody which recognizes the modified form of said motif;

(b) reacting an enzyme sample with a known substrate comprising the unmodified form of said motif; and

(c) screening with said antibody of step (a) for the presence of modified substrate resulting from step(b), thereby identifying if said enzyme modifies said known substrate.

31. (withdrawn) A method for profiling protein levels or post-translational modifications in a cell or tissue on a genome wide scale, said method comprising the steps of:

(a) generating at least one motif-specific, context-independent antibody which recognizes the modified form of a motif common to a plurality of proteins within a genome;

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- (b) preparing an extract of a cell or tissue; and
- (c) utilizing said antibody of step (a) to detect one or more proteins in the extract of step (b) that contain said modified motif, thereby profiling protein levels or post-translational modifications in said cell or tissue on a genome-wide scale.
32. (withdrawn) A method for profiling drug-induced changes in protein levels or post-translational modifications in a cell or tissue on a genome wide scale, said method comprising the steps of:
- (a) generating at least one motif-specific, context-independent antibody which recognizes the modified form of a motif common to a plurality of different proteins within a genome;
- (b) preparing an extract of a cell or tissue treated with said drug; and
- (c) utilizing said antibody of step (a) to profile drug-induced changes in the level or post-translational modification of one or more proteins containing said modified motif present in the extract of step (b) as compared against an untreated control extract.
33. (withdrawn) The method of any one of claims 27-32, wherein said motif is selected from the group consisting of a single phosphothreonine, a single phosphoserine, a single phosphotyrosine, a single acetyl-lysine, and a single nitrotyrosine.
34. (withdrawn) The method of any one of claims 27-32, wherein said motif comprises all or part of a kinase consensus substrate motif or a protein binding motif.
35. (withdrawn) The method of any one of claims 27-32, wherein said motif is selected from the group consisting of all or part of a MAPK consensus substrate motif, a CDK consensus substrate motif, a PKA consensus substrate motif, an Akt consensus substrate motif, a PKC consensus substrate motif, an ATM consensus substrate motif, a 14-3-3 consensus binding motif, a PDK1/bulky-ring consensus docking motif, and phosphothreonine-X-(R/K).
36. (withdrawn) The method of any one of claims 27-32, wherein said motif is selected from the group consisting of all or part of a PKC Zeta consensus substrate motif, a ABL kinase consensus substrate motif, a CDK5 consensus substrate motif, an insulin receptor consensus substrate motif, a CaMKII consensus substrate motif, a Src kinase consensus substrate motif, a CDC2/CDK2 consensus substrate motif, a GSK3 kinase consensus substrate motif, a PI3K P85 consensus binding motif, and proline-phosphoserine-proline.

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37. (withdrawn) A motif-specific, context-independent antibody which recognizes a plurality of peptides or proteins within a genome that contain said motif, wherein said motif comprises two to six invariant amino acids including at least one phosphotyrosine.
38. (withdrawn) A motif-specific, context-independent antibody which recognizes a plurality of peptides or proteins within a genome that contain said motif, wherein said motif comprises one to six invariant amino acids including at least one phosphoserine but does not consist of phosphoserine-proline.
39. (withdrawn) A motif-specific, context-independent antibody which recognizes a plurality of peptides or proteins within a genome that contain said motif, wherein said motif comprises two to six invariant amino acids including at least one phosphothreonine.
40. (withdrawn) The antibody of any one of claims 37-39, wherein said motif comprises all or part of a kinase consensus substrate motif or a protein binding motif.
41. (withdrawn) A motif-specific, context-independent antibody which recognizes a plurality of peptides or proteins within a genome that contain said motif, wherein said motif consists of a single phosphothreonine, and wherein the specificity of said antibody is independent of variations in the amino acid residue at the -1 position relative to said motif, as determined by peptide grid analysis.
42. (withdrawn) A motif-specific, context-independent antibody which recognizes a plurality of peptides or proteins within a genome that contain said motif, wherein said motif is selected from the group consisting of all or part of an MAPK consensus substrate motif, a CDK consensus substrate motif, and a 14-3-3 consensus binding motif.
43. (withdrawn) A motif-specific, context-independent antibody which recognizes a plurality of peptides or proteins within a genome that contain said motif, wherein said motif is selected from the group consisting of all or part of an Akt consensus substrate motif, a PKA consensus substrate motif, and a bulky-ring/PDK1 consensus docking motif.
44. (withdrawn) A motif-specific, context-independent antibody which recognizes a plurality of peptides or proteins within a genome that contain said motif, wherein said motif is selected from the group consisting of all or part of a PKC consensus substrate motif or an ATM consensus substrate motif, and phosphothreonine-X-(R/K).

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45. (withdrawn) A motif-specific, context-independent antibody which recognizes a plurality of peptides or proteins within a genome that contain said motif, wherein said motif is selected from the group consisting of all or part of a PKC Zeta consensus substrate motif, an ABL consensus substrate motif, a CDK5 consensus substrate motif, an insulin receptor consensus substrate motif, a PI3K P85 consensus binding motif, a CaMKII consensus substrate motif, a Src kinase consensus substrate motif, a CDC2/CDK2 consensus substrate motif, a GSK3 kinase consensus substrate motif, and proline-(phosphoserine/phosphothreonine)-proline.